W1
CA547

March 1995 Volume 11 Number 3

THE CANADIAN JOURNAL OF CARDIOLOGY

JOURNAL CANADIEN DE CARDIOLOGIE

The Journal of the Canadian Cardiovascular Society
Journal de la Société canadienne de cardiologie

PULSUS GROUP INC

Applicants: David J. Pinsky
U.S. Serial No.: 09/374,586
Filed: August 13, 1999
Group Art Unit: 1633

Exhibit 4

Individual variation in the effects of ASA on platelet function: Implications for the use of ASA clinically

MICHAEL R BUCHANAN PhD, STEPHANIE J BRISTER MD FRCS

MR BUCHANAN, SJ BRISTER. Individual variation in the effects of ASA on platelet function: Implications for the use of ASA clinically. Can J Cardiol 1995;11(3):221-227.

OBJECTIVE: To determine whether acetylsalicylic acid (ASA) inhibits hemostasis and platelet function in some individuals (ASA responders) but not in others (ASA nonresponders).
DESIGN: In this two-part study, part 1 was a randomized, double-blind crossover study of the effects of various single doses of ASA (80 to 1300 mg) on primary hemostasis and platelet function. Part 2 was a prospective cohort study of the effects of a chronic dose of ASA (325 mg) on primary hemostasis and platelet function.
SETTING: A hospital research laboratory and a cardiac care ward.
SUBJECTS: Part 1: 10 healthy volunteers (five male, five female). Part 2: 40 consecutive patients undergoing elective coronary artery bypass grafting (CABG).
RESULTS: Part 1: ASA, in a dose-related manner, prolonged the bleeding time in 60% of volunteers (ASA responders), which was associated with decreases in platelet thromboxane (Tx) A_2 and 12-hydroxyeicosatetraenoic acid (12-HETE) synthesis and in platelet aggregation and adhesion. However, in volunteers whose bleeding time was not prolonged (ASA nonresponders), platelet 12-HETE synthesis and platelet adhesion were unchanged or increased ($P<0.001$), despite platelet TxA_2 and platelet aggregation being inhibited. Part 2: similarly, 58% of the CABG patients were ASA responders and all of their platelet biochemistry and function tests were inhibited, while in the CABG patient ASA nonresponders (no prolongation of bleeding time), platelet 12-HETE and platelet adhesion were increased ($P<0.001$).

continued on next page

Presented in part at the Canadian Cardiovascular Society meeting, Vancouver, British Columbia, October 25, 1993
Departments of Pathology and Surgery, McMaster University and Hamilton Civic Hospitals, General Division, Hamilton, Ontario
Correspondence and reprints: Dr MR Buchanan, 293 Wellington Street, Suite 132, Hamilton, Ontario L8L 8E7. Telephone (905) 521-9795, Fax (905) 523-4885
Received for publication May 12, 1994. Accepted July 26, 1994

ACETYLSALICYLIC ACID (ASA) IS THE antithrombotic agent of choice for the prevention and treatment of disorders associated with arterial thrombosis (1-9). Meta-analysis of more than 180 clinical trials suggests that, overall, ASA reduces the risks of stroke, myocardial infarction and vascular death by 25% in patients with various cardiovascular diseases (7,9).

ASA is given to patients as an antiplatelet drug on the basis that its acetyl moiety inactivates platelet cyclooxygenase irreversibly, thereby preventing platelet thromboxane A_2 (TxA_2) synthesis and rendering platelets less responsive to hemostatic and thrombogenic stimuli (10,11).

Not all patients who are treated with ASA derive benefit from the drug, ie, some patients still suffer a thromboembolic event despite ASA therapy. There are a number of possible explanations for these failures. First, the dose of ASA administered to some patients may be too low (2,5,12,13). This explanation forms the basis for ongoing debates concerning the optimal dose of ASA. Second, others have suggested that some patients are resistant to ASA, ie, ASA impairs platelet function in some patients (ASA responders) but not in others (ASA nonresponders) (14-16). The underlying mechanism to ex-

CONCLUSIONS: ASA does not impair platelet function and related hemostasis in a significant percentage of individuals. These individuals may not derive the same benefits from ASA therapy.

Key Words: *12-HETE, Acetylsalicylic acid, Bleeding time, Platelet adhesion, Thrombosis*

Variation interindividuelle des effets de l'AAS sur la fonction plaquettaire : répercussions sur l'utilisation clinique de l'AAS

OBJECTIF : Déterminer si l'acide acétylsalicylique (AAS) inhibe différemment l'hémostase et la fonction plaquettaire selon que les individus répondent à l'AAS ou non.

MODÈLE : Dans cette étude en deux volets, le volet 1 a été un essai croisé, à double insu, randomisé, sur les effets de diverses doses d'AAS (80 à 1 300 mg) sur l'hémostase primaire et la fonction plaquettaire. La partie 2 a été une étude de cohorte prospective sur les effets de l'administration continue d'AAS (325 mg) sur l'hémostase primaire et la fonction plaquettaire.

CONTEXTE : Unité de laboratoire de recherche et unité de cardiologie d'un hôpital.

SUJET : Partie 1 : 10 volontaires en santé (5 hommes, 5 femmes). Partie 2 : 40 patients consécutifs ayant subi un pontage aorto-coronarien non urgent (PAOC).

RÉSULTATS : Partie 1 : l'AAS, selon un modèle lié à la dose, a prolongé le temps de saignement chez 60 % des volontaires (qui répondaient à l'AAS) qui a été associé à une diminution de la synthèse de la thromboxane A_2 et de l'acide 12-hydroxyeicosatétraénoïque (12-HETE) plaquettaire et de l'agrégation et de l'adhésivité plaquettaires. Toutefois, chez les volontaires dont le temps de saignement n'a pas été prolongé (qui ne répondaient pas à l'AAS), la synthèse du 12-HETE plaquettaire et l'adhésivité plaquettaire sont restées inchangées ou ont augmenté ($P<0,001$), malgré l'inhibition de la TxA_2 plaquettaire et de l'agrégation plaquettaire. Partie 2 : de même, 58 % des patients ayant subi un PAOC ont répondu à l'AAS et toutes leurs valeurs plaquettaires et les épreuves de fonction ont été inhibées, alors que chez les patients ayant subi un PAOC et qui ne répondaient pas à l'AAS, aucun prolongement du temps de saignement, le 12-HETE plaquettaire et l'agrégation plaquettaire ont été augmentés ($P<0,001$).

CONCLUSIONS : L'AAS n'altère pas la fonction plaquettaire et l'hémostase chez un important pourcentage de sujets. Ces sujets risquent de ne pas tirer le même avantage d'un traitement par AAS.

plain these latter differences has not been elucidated, nor have the clinical consequences of being an ASA responder or ASA nonresponder been identified. A third possibility that has been alluded to in some experimental studies (17-19) is that ASA treatment may be associated with an increased risk of thromboembolic events in some individuals. This possibility has not been taken seriously because of the abundant evidence that ASA has an overall 'net' benefit (1-9), and because there has been neither a clear explanation for, nor an identification of an underlying mechanism of action to explain, such an effect. This notwithstanding, there are some recent clinical reports suggesting that ASA may exacerbate thrombosis in some individuals (20-22).

The thrombogenic effects of ASA seen in the experimental setting were attributed initially to an inhibition of vessel wall cell cyclooxygenase and subsequent prostacyclin inhibition. More recent data, however, provide an alternative explanation. It is well documented that ASA acetylates cyclooxygenase, thereby preventing the metabolism of arachidonic acid to TxA_2, a potent platelet agonist. Inhibition of TxA_2 is associated with impaired platelet aggregation (10,11,13). However, arachidonic acid is also metabolized simultaneously by another platelet enzyme, lipoxygenase, into the monohydroxide, 12-hydroxyeicosatetraenoic acid (12-HETE) (23,24). Moreover, when platelet cyclooxygenase is inhibited, platelet 12-HETE synthesis via the lipoxygenase pathway may increase (25-28). The increase in 12-HETE synthesis is associated with an increase in platelet adhesivity (26,27). Other studies suggest that 12-HETE facilitates platelet adhesion by altering the ability of glycoprotein-like adhesion receptors to recognize their adhesion ligands (29,30).

If increased platelet adhesion is clinically relevant, the latter observations are consistent with the possibility that ASA can enhance overall platelet reactivity in some individuals, thereby increasing their risk of thromboembolic events. Therefore, we set out to determine, in both healthy volunteers and patients undergoing elective coronary artery bypass grafting (CABG), whether ASA has differing effects on platelet arachidonic acid metabolism and platelet function in specific individuals ex vivo and, if so, whether there is any relationship between these differences and platelet hemostatic function in vivo.

PATIENTS AND METHODS

Both studies (study 1 in healthy volunteers and study 2 in CABG patients) were approved by the Institutional Review Board at the Hamilton Civic Hospitals. All volunteers signed an approved informed consent form. The studies were performed between September 1991 and March 31, 1993 at the Hamilton General Hospital.

Study 1: Study 1 was a double-blind, randomized study to determine the effects of varying doses of ASA on prolongation of bleeding time, on platelet TxA_2 and 12-HETE synthesis, and on platelet aggregation and adhesion. Ten healthy volunteers (five males and five females between the ages of 20 and 42 years, mean ± SD 30±7 years) were recruited for this study. The inclusion criteria were apparent good health with no cardiovascular disease signs or symptoms. All volunteers gave informed consent. The exclusion criteria were a history of bleeding, receipt of an experimental drug within 30 days before this study, known hypersensitivity to ASA,

any psychiatric problem, and either pregnancy or intention to become pregnant within 40 days of the study. Volunteers were requested to follow their regular diets and to abstain from ingesting any nonsteroidal anti-inflammatory drug throughout the study. Finally, volunteers were required to fast for 12 h before each test day. All volunteers who were initially recruited completed the study.

On day 1, 20 mL of whole blood was collected from an antecubital vein of each volunteer via an 18 gauge thin wall needle. A Surgicutt II bleeding time test (31), duplicate cuts, was performed in the opposite arm. Each volunteer was then given an envelope containing one of five ASA doses assigned randomly (80, 160, 325, 650 or 1300 mg of ASA [Bayer, Sterling Winthrop]). Each ASA dose was ingested orally with 100 mL of water. Two hours later, a second Surgicutt II bleeding time test was performed and a second 20 mL blood sample was collected. This procedure was repeated four times at 14-day intervals until all volunteers had been tested with each ASA dose. A 14-day washout period between doses to avoid any residual carryover effect of ASA was considered adequate since ASA is rapidly hydrolyzed in vivo (half-life about 15 mins) and the irreversible inhibitory effect of ASA on platelet function is completely overcome within seven to 10 days with the turnover of new platelets (10,11,13).

Study 2: Study 2 was a prospective cohort study in 40 consecutive patients (31 males and seven females aged 60±8 and 60±6 years, mean ± SD, respectively) undergoing elective CABG who met the study's inclusion and exclusion criteria. Included were any adult patient who had agreed to undergo elective CABG and any patient who was ingesting 325 mg ASA daily and had been doing so for at least six months before surgery. Patients were chosen who had been ingesting ASA for at least six months to ensure that those who were ingesting ASA chronically were included. All patients gave informed consent. Excluded from the study were any patient with a history of bleeding complications, any patient who had received any experimental drug within 30 days before the study, any patient who had a recent (within one year) history of alcoholism or drug addiction, any patient known to be hypersensitive to ASA, any patient with a psychiatric problem, all insulin-dependent diabetic patients, any patient who was unable to discontinue ASA or any other nonsteroidal anti-inflammatory drug seven days before surgery and any patient with a platelet count less than $1x10^9$/L.

Thirty days before surgery, a 20 mL blood sample was collected from each patient and a Surgicutt II bleeding time test was performed, as described for study 1. At that time, all patients attested that they were ingesting 325 mg of enteric-coated ASA daily and had ingested the last ASA tablet about 2 h beforehand. Seven days before surgery, each patient was contacted by telephone and asked to stop taking ASA. Seven days later, immediately before surgery, a second 20 mL blood sample was collected from the patient and a second Surgicutt II bleeding time test was performed. This latter bleeding time test was performed in addition to a Surgicutt I bleeding time test (which is performed routinely in every CABG patient) by laboratory technologists not involved in this study.

Compliance of all volunteers and patients in regard to ASA ingestion was confirmed by comparing the platelet TxA_2 levels in the samples collected from each individual while on and off ASA. All 40 patients completed the study.

Platelet function and biochemistry assays: Blood samples collected from the healthy volunteers and CABG patients were centrifuged at 180 *g* for 15 mins at 37°C to prepare platelet-rich plasma. The platelet count was adjusted to $2x10^9$ platelets/L using autologous platelet-poor plasma. ADP- and collagen-induced platelet aggregation was measured over 5 mins via the optical density turbidity assay (32). A 100 µL aliquot of each aggregating sample was then decanted into an Eppendorf tube and centrifuged at 10,000 *g* for 15 s to prepare platelet-poor plasma, which was stored at –70°C until assayed for TxA_2. TxA_2 (measured as TxB_2) was determined via a specific radioimmunoassay (33).

Fifteen minutes later, the remaining aggregating sample was transferred to a silanized vial containing an equivolume of chloroform and extracted for 12-HETE (34). 12-HETE was measured by high performance liquid chromatography, as previously described (34).

Aliquots (2 mL) of each platelet-rich plasma were also incubated with ^{3}H-adenine for 40 mins at 37°C to label the platelets. Three aliquots (500 µL each) of the ^{3}H-adenine-labelled platelets were then exposed to fibronectin-coated discs in Costar 24 culture wells (Fisher Scientific) for 30 mins. Each disc was removed from the platelet-rich plasma and rinsed three times in phosphate-buffered saline to remove nonadherent platelets. The ^{3}H-radioactivity of each disc was determined by beta liquid scintillation counting. The number of platelets adherent to each disc was calculated on the basis of disc radioactivity and ^{3}H-platelet specific activity (35).

Data handling and analysis: It was assumed that if any dose of ASA had a potential antithrombotic effect, then platelet function should be impaired. Consequently, each individual (volunteer or patient) should be hemostatically defective, and defective hemostasis should be reflected by a prolonged bleeding time. If, on the other hand, any specific dose of ASA did not have a potential antithrombotic effect, then hemostasis should be unchanged and the bleeding time should not be significantly prolonged. Therefore, both the healthy volunteers and patients were defined as ASA responders or ASA nonresponders as follows: individuals whose bleeding time was prolonged by ASA more than 2 SD from their bleeding time measured while off ASA were defined as ASA responders, irrespective of ASA dose and whether they had been defined as ASA responders or ASA nonresponders at any other ASA dose. Individuals whose bleeding time was not prolonged by ASA, ie, less than 2 SD from their bleeding time while off ASA, were defined as ASA nonresponders. (Two SD was used since the 2 SD of five pre-ASA bleeding time val-

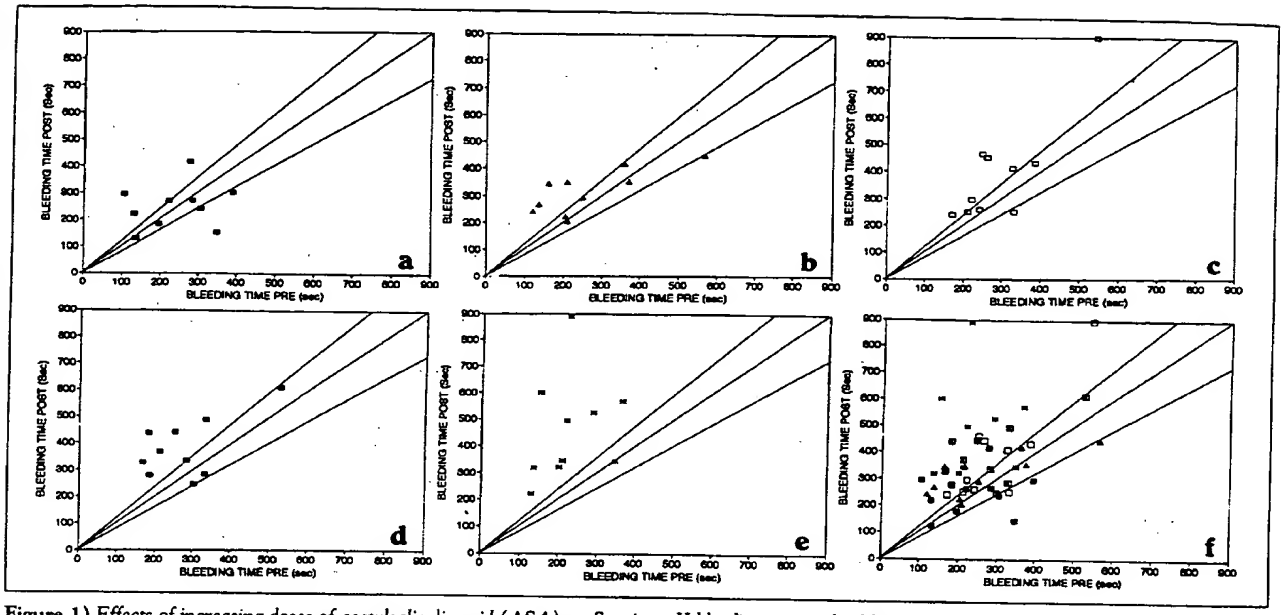

Figure 1) *Effects of increasing doses of acetylsalicylic acid (ASA) on Surgicutt II bleeding time in healthy volunteers. Data points of individuals whose post-ASA bleeding times lay above the upper line (+2 SD) were defined as ASA responders. Data points of individuals whose post-ASA bleeding times lay within or below the upper and lower lines (±2 SD) were defined as ASA nonresponders. Data are represented for volunteers ingesting* a *80 mg,* b *160 mg,* c *325 mg,* d *650 mg,* e *1300 mg and* f *all doses of ASA*

ues within individuals, measured in the volunteer group at two-week intervals by the same assessor, was ±19%, and the 2 SD in bleeding time values among individuals as measured by three independent assessors in the CABG patient group was ±21%.) Healthy volunteer and CABG patient ASA responders and ASA nonresponders were subgrouped separately. All data were analyzed by ANOVA, linear regression or a Mann-Whitney nonparametric test via a computer-assisted MINITAB program (Release 7, Minitab Inc, Data Tech Industries, Pennsylvania).

RESULTS

Study 1 – healthy volunteers, single-dose ASA treatment: When the 10 volunteers ingested 80 mg of ASA, the bleeding time was prolonged more than 2 SD in only three individuals (Figure 1a). As the dose of ASA was increased up to 1300 mg, the number of volunteers whose bleeding times were prolonged increased progressively (Figure 1a to 1e). For example, only three of the 10 volunteers had prolonged bleeding times and were defined as ASA responders after ingesting 80 mg of ASA, while nine volunteers had prolonged bleeding times after ingesting 1300 mg of ASA and were defined as ASA responders to that dose (Figure 1e). Thus, most volunteers who were ASA nonresponders to the low doses of ASA were responders to the highest dose of ASA, ie, prolongation of the bleeding time was ASA dose-dependent, r=0.9900, P<0.001.

To determine whether the inhibitory effect(s) of ASA on platelet biochemistry and platelet function varied between ASA responders and ASA nonresponders, both among different individuals and within the same individual, platelet biochemistry and platelet function data of ASA responders were compared with those of ASA nonresponders, irrespective of dose, since some individuals were ASA nonresponders at some doses but became ASA responders at higher doses (Figure 1f).

There were no differences in the extent of inhibition of ADP- or collagen-induced platelet aggregation between the ASA responders and ASA nonresponders (data not shown). Inhibition of platelet aggregation was associated with a significant inhibition of TxA_2 in all volunteers, P<0.001, irrespective of their being ASA responders (–77±12%) or ASA nonresponders (–74±12%) or of ASA dose.

In contrast, ASA had markedly different effects on platelet adhesivity and platelet 12-HETE synthesis in the ASA responders compared with the ASA nonresponders. In ASA responders, platelet adhesivity decreased by about 30±8% after ASA treatment (Figure 2). The inhibition of platelet adhesivity was associated with a 57±7% inhibition of platelet 12-HETE synthesis (Figure 2). In nonresponders, both platelet adhesivity and platelet 12-HETE increased after ASA, by 31% and 10%, respectively (Figure 2). The differences between decreased platelet adhesivity and platelet 12-HETE in responders and increased adhesivity and 12-HETE in nonresponders were significant (P<0.0008). Moreover, the changes in bleeding were inversely correlated with the changes in platelet adhesivity (r=0.4320, P<0.005).

Study 2 – CABG patients, chronic ASA treatment: Similar results were observed in the CABG patients. In 23 of the 40 patients, bleeding times were significantly prolonged when patients

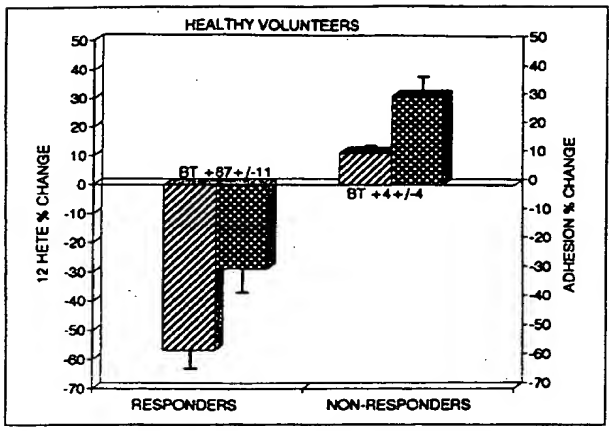

Figure 2) *Effects of acetylsalicylic acid on platelet 12-hydroxyeicosatetraenoic acid (12-HETE) synthesis (hatched bars) and platelet adhesivity (crosshatched bars) in healthy volunteers. Platelet 12-HETE synthesis and platelet adhesivity in the 10 volunteers when their bleeding times were prolonged (+87±11%, 31 measures at the various doses) are shown on the left. Platelet 12-HETE synthesis and platelet adhesion in the 10 volunteers when their bleeding times were unchanged (+4±4%, 19 measures at the various doses) are shown on the right*

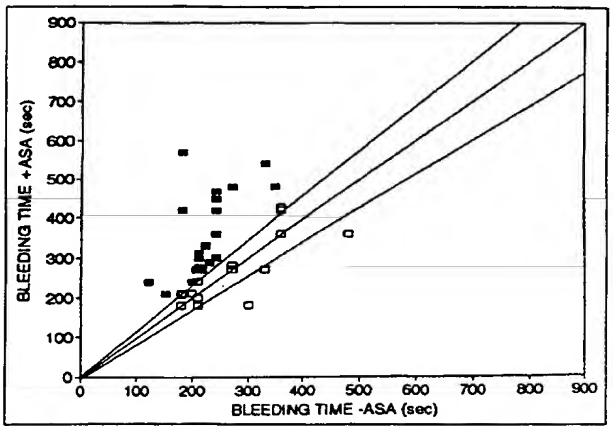

Figure 3) *Effects of chronic acetylsalicylic acid (ASA) ingestion (325 mg daily) on the Surgicutt II bleeding time in patients undergoing elective coronary artery bypass grafting. All patients whose bleeding times fell above the +2 SD line while on ASA (solid squares, n=23) were defined as ASA responders. All patients whose bleeding times fell within or below the +2 SD lines while on ASA (open squares n=17) were defined as ASA nonresponders*

were ingesting 325 mg of ASA daily on a routine basis (Figure 3). In 17 of the 40 patients, bleeding times were unchanged or shortened while they were taking ASA. There were no significant differences in the inhibitory effects of ASA on ADP- or collagen-induced platelet aggregation (data not shown) or on inhibition of platelet TxA_2 synthesis (ASA responders, –74±12%; ASA nonresponders, –78±12%).

In patients whose bleeding times were significantly prolonged (ASA responders, bleeding times increased 58±10%), platelet adhesivity decreased by 29±8%. This decrease was associated with a 56±12% reduction in 12-HETE synthesis (Figure 4). In contrast, in patients whose bleeding times were not prolonged (ASA nonresponders, only a 2±4% change in bleeding time), both platelet 12-HETE and platelet adhesivity increased. The differences in decreased platelet adhesion and 12-HETE in the ASA responders, and in increased platelet adhesion and 12-HETE in the ASA nonresponders, were significant, P<0.0005. Moreover, the changes in bleeding times were inversely correlated with the changes in platelet adhesivity, r=0.4510, P<0.005.

Finally, the reliability of the bleeding time measurements was confirmed by comparing the presurgery bleeding time values measured by the researchers with those obtained by the routine laboratory technologists. The correlation between the measurements was r=0.9206, P<0.0001.

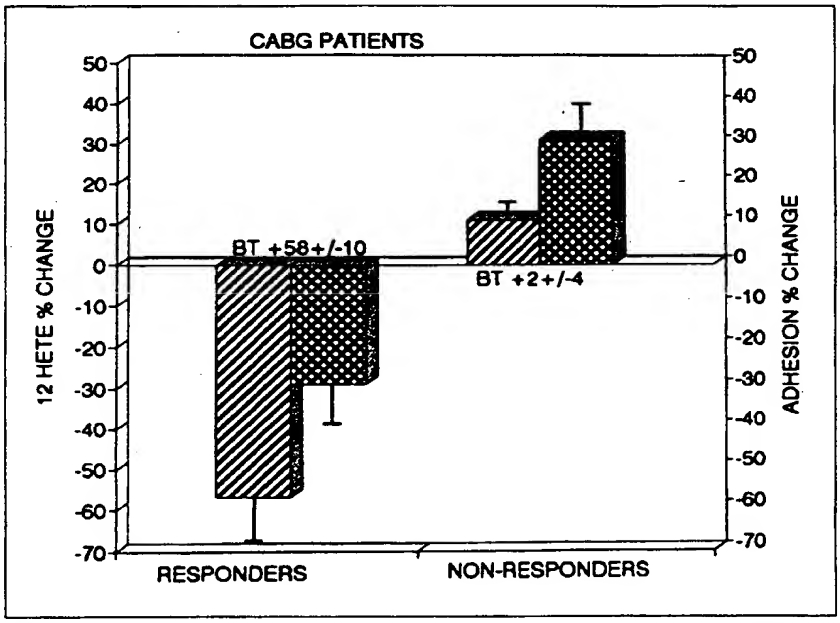

Figure 4) *Relative effects of acetylsalicylic acid (ASA) on platelet 12-hydroxyeicosatetraenoic acid (12-HETE) synthesis (hatched) and platelet adhesivity (crosshatched) in coronary artery bypass graft (CABG) patients classified as ASA responders (left, n=23) and ASA nonresponders (right, n=17). Data are expressed as mea n± SD. Bleeding times (BT) are expressed as percentage change in bleeding time when on ASA compared with the patients' immediate preoperative bleeding time when off ASA for seven days*

DISCUSSION

We have demonstrated that ASA, in doses varying from 80 to 1300 mg and ingested either as a single dose (by healthy volunteers) or chronically (by cardiovascular diseased patients), has varying effects on platelet function and hemostasis. Specifically, ASA renders some individuals (responders) hemostatically defective, ie, ASA impairs both platelet TxA_2 and 12-HETE synthesis, thereby inhibiting platelet aggregation and adhesion and prolonging bleeding time. However, ASA does not render other individuals (or the same individual given a lower ASA dose) hemostatically defective (nonresponders). In those individuals, platelet 12-HETE synthesis and platelet adhesivity remain unchanged or become enhanced, even when platelet TxA_2 and platelet aggregation are impaired to the same extent as the ASA responders. The former changes are reflected by the bleeding time not being prolonged.

These results have a number of implications concerning the clinical use of ASA as an antiplatelet drug. First, the observation that subgrouping healthy volunteers and CABG patients on the basis of bleeding time response to ASA also results in subgrouping of the same individuals with respect to specific effects of ASA on platelet function suggests that the bleeding time test is a useful screening tool to discriminate between the two individual groups, ie, to discriminate ASA responders from nonresponders. The good reproducibility of the bleeding time measure is also supported by the high correlation between the bleeding time measures made by different individuals.

Second, the observations that prolonged bleeding times (defective hemostasis) are paralleled by decreases in both platelet TxA_2 and 12-HETE synthesis as well as corresponding decreases in both platelet aggregation and adhesivity in responders are consistent with earlier studies suggesting that platelet 12-HETE and adhesivity are important in hemostasis and thrombosis (25-28,30,35). This is further supported by the observations that when bleeding times remained unchanged (or shortened) in the ASA nonresponders, there were corresponding changes in platelet 12-HETE and platelet adhesivity, despite platelet TxA_2 and aggregation being inhibited.

The explanation for these differential effects of ASA on platelet 12-HETE synthesis and subsequent platelet adhesivity is not known. Meade et al (36) recently reported that COS cells contain two cyclooxygenase isozymes. One isozyme (prostaglandin H synthase 1) is constitutive and readily inhibitable by ASA. The second isozyme (prostaglandin H synthase 2) is synthesized de novo following stimulation and is resistant to inhibition by ASA. Moreover, the second isozyme is capable of synthesizing arachidonic acid to 12-HETE (36,37). Other investigators have suggested that both isozymes are normally coexpressed in most human tissue (38). If platelets also have these two cyclooxygenase isozymes, it is possible that the relative levels of these isozymes differ in platelets of ASA responders and nonresponders, such that platelets of ASA nonresponders contain more prostaglandin H synthase 2. This could explain the lack of an effect of ASA in 12-HETE synthesis in those individuals. Alternatively, these differential ASA effects may be related to differential effects of the salicylate moiety of ASA in responders and nonresponders. This moiety has been shown to impair the lipoxygenase pathway independently of the acetylation of platelet cyclooxygenase. As a consequence, 12-HETE synthesis is blocked, rendering platelets less adhesive (26,27,30,35). It is possible that the acetyl and salicylate moieties of ASA affect responder and nonresponder platelets differently, depending on the sensitivities of the cyclo- and lipoxygenase peroxidases to ASA. Whichever possibility is real, the fact remains that ASA has different effects on platelets in different individuals. These effects are only, in part, related to the ASA dose, as demonstrated in our volunteer study.

Third, the differential effects of ASA on the lipoxygenase pathway in responders and nonresponders may explain the apparent failure of ASA to provide a protective effect in some clinical studies. For example, Grotemeyer et al (20) demonstrated that ASA responders benefit more from ASA treatment than do nonresponders. Specifically, they demonstrated that only 4% of computed tomography scan-positive stroke patients (who were subgrouped as 'ASA responders') suffered a second thrombotic event within the subsequent two years of the initial event, whereas 40% (subgrouped as 'ASA nonresponders'), suffered a second thrombotic event while on ASA. While these investigators could not conclude that ASA actually increased these thrombotic events because a placebo control group was not included in that study, other clinical data suggest that the two-year thrombotic event rate in similar patient populations is only about 25% (5,22). It is possible that ASA had a more complete inhibitory effect on platelet function in Grotemeyer's ASA responders, thereby affording those patients a beneficial antithrombotic effect, whereas ASA failed to impair platelet adhesion in the ASA nonresponder patients, thereby providing them with no beneficial effect.

ASA may not afford all patients a beneficial antiplatelet effect since ASA does not exert the same 'antiplatelet effect' in all individuals. We also suggest that the Surgicutt II bleeding time may be a more reliable test to identify those individuals who will benefit most from ASA therapy, ie, the ASA responders. This possibility is being tested in a Canadian multicentre trial (Benefits and Risks of ASA in Thrombosis [BRAT] study), which commenced May 15, 1994.

REFERENCES

1. The Canadian Cooperative Study Group. A randomized trial of aspirin and sulfinpyrazone in threatened stroke. N Engl J Med 1978;299:53-9.
2. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high grade carotid stenosis. N Engl J Med 1991;325:445-53.
3. The Steering Committee of the Physicians' Health Study Research Group. Preliminary report: findings from the aspirin component of the ongoing Physicians' Health Study. N Engl J Med 1988;318:262-4.

4. Ciabattoni G, Ujang S, Sritara P, Andreotti F, Davies G, Simonetti BM. Aspirin, but not heparin, suppresses the transient increase in thromboxane biosynthesis associated with cardiac catheterization or coronary angioplasty. J Am Coll Cardiol 1993;21:1377-81.
5. Boysen G, Sorensen PS, Juhler M. Danish very-low-dose aspirin after carotid endarterectomy trial. Stroke 1988;19:1211-5.
6. Johnson WD, Kayser KL, Hartz AJ, Saedi SF. Aspirin use and survival after coronary bypass surgery. Am Heart J 1992;123:603-8.
7. Aspirin Trialists Collaboration. Secondary prevention of vascular disease by prolonged antiplatelet treatment. BMJ 1988;296:320-31.
8. The RISC Group. Risk of myocardial infarction and death during treatment with low dose aspirin and intravenous heparin in men with unstable coronary artery disease. Lancet 1990;336:827-30.
9. Roux S, Christeller S, Ludin E. Effects of aspirin on coronary reocclusion and recurrent ischemia after thrombolysis: A meta-analysis. J Am Coll Cardiol 1992;19:671-6.
10. Roth GJ, Majerus PW. The mechanism of the effect of aspirin on human platelets. Acetylation of a particulate fraction protein. J Clin Invest 1975;56:624-32.
11. Burch JW, Stanford N, Majerus PW. Inhibition of platelet prostaglandin synthetase by oral aspirin. J Clin Invest 1978;61:314-9.
12. Ranke C, Hecker H, Creutzig A, Alexander K. Dose-dependent effect of aspirin on carotid atherosclerosis. Circulation 1993;87:1873-9.
13. Mohri H, Ohkubo T. Single-dose effect of enteric-coated aspirin on platelet function and thromboxane generation in middle-aged men. Ann Pharmacother 1993;27:405-10.
14. Doutremepuich C, Pailley D, Anne MC, de Seze O, Paccalin J, Quilichini R. Template bleeding time after ingestion of ultra low dosages of acetylsalicylic acid in healthy subjects. Preliminary study. Thromb Res 1987;48:501-4.
15. Doutremepuich C, de Seze O, LeRoy D, Dalanne MC, Anne MC. Aspirin at very ultra low dosage in healthy volunteers: Effects on bleeding time, platelet aggregation and coagulation. Hemostasis 1990;20:99-105.
16. Juergens UR, Christiansen SC, Stevenson DD, Zuraw BL. Arachidonic acid metabolism in monocytes of aspirin-sensitive asthmatic patients before and after oral aspirin challenge. J Allergy Clin Immunol 1992;90:636-45.
17. Zimmermann R, Thiessen M, Walter E, Morl H. Paradoxical effect of high mid low dose aspirin in experimental arterial thrombosis. Artery 1980;8:422-5.
18. Kelton JG, Hirsh J, Carter CJ, Buchanan MR. Thrombogenic effect of high-dose aspirin in rabbits. Relationship to inhibition of vessel wall synthesis of prostaglandin I2-like activity. J Clin Invest 1978;62:892-5.
19. Buchanan MR, Dejana E, Gent M, Mustard JF, Hirsh J. Enhanced platelet accumulation onto injured carotid arteries in rabbits following aspirin treatment. J Clin Invest 1981;67:503-8.
20. Grotemeyer K-H, Scharafinski H, Husstedt I-W. Two year follow-up of aspirin responder and aspirin non-responder: A pilot study including 180 post-stroke patients. Thromb Res 1993;71:397-403.
21. Ridker PM, Manson JE, Gaziano JM, Buring JE, Hennekens CH. Low-dose aspirin therapy for chronic stable angina. Ann Intern Med 1991;114:835-9.
22. Harker LA, Bernstein EF, Dilley RB, et al. Failure of aspirin plus dipyridamole to prevent restenosis after carotid endartertectomy. Ann Intern Med 1992;116:731-6.
23. Aharony D, Smith JB, Silver MJ. Platelet arachidonate lipoxygenase. In: Chakrin LW, Bailey DM, eds. The Leukotrienes. New York: Academic Press, 1984:104-23.
24. Schafer AI, Turner NA, Handin RI. Platelet lipoxygenase-dependent oxygen burst. Evidence for differential activation of lipoxygenase in intact and disrupted human platelets. Biochem Biophys Acta 1982;712:535-41.
25. Morita I, Murota SI. Role of 12-lipoxygenase products of arachidonic acid on platelet aggregation. Adv Prostaglandin Thromboxane Leukot Res 1987;17:219-23.
26. Buchanan MR, Butt RW, Hirsh J, Markham B, Nazir DJ. Role of lipoxygenase metabolism in platelet function: effect of aspirin and salicylate. Prostaglandin Leukot Med 1986;21:157-68.
27. Gibson BES, Buchanan MR, Barr RD, White JG. Primary thrombocythaemia in childhood: symptomatic episodes and their relationship to thromboxane A_2, 6-keto-PGE_1 and 12-hydroxy-eicosatetraenoic acid production: a case report. Prostaglandin Leukot Med 1987;26:221-31.
28. Setty BNY, Werner MH, Hannun YA, Stuart MJ. 15-hydroxyeicosatetraenoic acid mediated potentiation of thrombin-induced platelet functions occur via enhanced production of phosphoinositide-derived second messengers – sn-1, 2-diacyclglycerol and inositol-1,4,5-trisphosphate. Blood 1992;80:2765-73.
29. Timar J, Chen YQ, Liu B, Bazaz R, Taylor JD, Honn KV. The lipoxygenase metabolite 12(S)-HETE promotes α-IIbβ_3 integrin-mediated tumor-cell spreading on fibrinectin. Int J Cancer 1992;52:594-603.
30. Buchanan MR, Bastida E. Endothelium and underlying membrane reactivity with platelets, leukocytes and tumor cells: Regulation by the lipoxygenase-derived fatty acid metabolites, 13-HODE and HETE's. Med Hypothesis 1988;27:317-25.
31. Buchanan GR, Holtkamp CA. A comparison of variables affecting the bleeding time using two disposable devices. Am J Clin Pathol 1989;91:45-50.
32. Mustard JF, Perry DW, Kinlough-Rathbone R, Packham MA. Factors responsible for ADP-induced release reaction of human platelets. Am J Physiol 1975;228:1757-65.
33. Buchanan MR, Rischke JA, Butt R, Turpie AGG, Hirsh J, Rosenfeld J. The sex-related differences in platelet function and aspirin pharmacokinetics in rabbits and man. Thromb Res 1983;29:125-39.
34. Haas TA, Buchanan MR. An automated extraction and quantification procedure for lipoxygenase metabolites. J Chromatogr 1988;430:1-10.
35. Buchanan MR, Butt RW, Turpie AGG. Effect of nafazatrom on platelet function and release: relationship to symptomatic episodes in patients with peripheral vascular disease. Am Heart J 1987;113:1133-7.
36. Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclo-oxygenase) isozymes by aspirin and other non-steriodal anti-inflammatory drugs. J Biol Chem 1993;268:6610-4.
37. Holtzman MJ, Turk J, Shornick LP. Identification of a pharmacologically distinct protaglandin H synthase in cultured epithelial cells. J Biol Chem 1992;267:21438-45.
38. O'Neill GP, Ford-Hutchinson AW. Expression of mRNA for cyclo-oxygenase-1 and cyclooxygenase-2 in human tissue. FEBS Lett 1993;330:156-60.